DOI 10.1007/s11055-019-00880-3
Neuroscience and Behavioral Physiology, Vol. 50, No. 2, February, 2020

The Role of the Stress Factor in Mediating the Genetic Predisposition to Stroke of the Background of Hypertensive Disease

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Translated from Zhurnal Nevrologii i Psikhiatrii imeni S. S. Korsakova, Vol. 119, No. 3, Iss. 2, Stroke, pp. 11–17, March, 2019. Original article submitted October 8, 2018. Accepted October 22, 2018.

Objectives. To study the interaction of polymorphic markers for matrix metalloproteinases (MMP) and chronic stress on formation of stroke on the background of hypertensive disease. **Materials and methods.** A total of 830 patients were studied: 303 patients with ischemic stroke on the background of hypertensive disease and 527 patients with hypertensive disease without stroke. SNP for metalloproteinases were studied using the real-time polymerase chain reaction. The functional significance and influences of polymorphic loci on gene expression were studied using the HaploReg (v4.1) (http://archive.broadinstitute.org) and GTEx-portal (http://gtexportal.org) services. **Results and conclusions.** An association was found between the GG rs11568818 genotype of the *MMP7* gene and a high risk of developing stroke in patients experiencing regular stress (odds ratio (OR) 1.71). The 5A allele and the 5A/5A genotype of rs3025058 of the *MMP3* gene had protective effects on development of stroke in people without histories of chronic stress (OR 0.73 and OR 0.60, respectively). The SNP studied here were located in the histone protein H3K4me1 and H3K4me3 region, which is hypersensitive to DNase 1 and binds regulatory proteins and transcription factors, while the polymorphic rs11568818 locus is linked with the level of expression of the *MMP7* gene.

Keywords: ischemic stroke, matrix metalloproteinases, single-nucleotide polymorphism, stress.

More than 400,000 cases of stroke are recorded in the Russian Federation each year, with death from cerebrovascular diseases being the highest among the European states [1]. Ischemic stroke is an acute impairment to cerebral blood flow, is accompanied by brain tissue hypoxia and necrosis, and leads to neurological deficit [2]. Stroke involves triggering of a cascade of neuroinflammatory reactions, the key element of which is release of matrix metalloproteinases (MMP), which are responsible for degrading components of the extracellular matrix [3]. Previous studies showed that the level of expression of *MMP* genes correlates with the development of cerebral stroke [4]. Along with genetic factors, environmental factors are also involved in the occurrence of stroke; among these, an important role is played by chronic emotional stress [5, 6]. Epidemiological studies show that long-lasting psychoemotional tension leads to changes in the biochemical composition and electrolyte composition of the blood, thrombus formation, and suppression of the immune system [6, 7]. The aim of the present work was to study the role of chronic stress in realizing the genetic predisposition to stroke on the background of hypertensive disease (HTD).

Materials and methods. Results from investigation of 830 patients were analyzed: group 1 consisted of 303 patients with ischemic stroke on the background of HTD; group 2 consisted of 527 patients with HTD without any history of impairment to the cerebral circulation. Groups were formed in the period from 2013 to 2016 in the Departments of Neurology and Cardiology, St. Joseph Belgorod District Clinical Hospital (BDCH). Among stroke patients, the atherothrombotic subtype was seen in 124 patients (40.93%), the cardioembol-

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TABLE 1. Clinical Characteristics of Study Groups of Patients

Parameter	Group 1 (n = 303)	Group 2 (n = 527)	p
Men/women, abs. (%)	201/102 (66.34/33.66)	322/205 (61.11/38.89)	0.15
Mean age, years	59.58 ± 8.21	58.81 ± 7.74	0.85
BMI, kg/m^2	30.64 ± 5.63	31.05 ± 4.43	0.09
Systolic BP, mmHg	190.73 ± 24.21	176.04 ± 16.28	0.001
Diastolic BP, mmHg	108.53 ± 13.42	103.83 ± 9.12	0.001
TCH, mM	5.73 ± 1.13	5.74 ± 1.07	0.92
HDL CH, mM	1.28 ± 0.31	1.38 ± 0.42	0.001
LDL CH, mM	3.76 ± 1.06	3.81 ± 0.73	0.35
TG, mM	1.83 ± 1.14	2.05 ± 0.65	0.001
Presence of chronic stressors, abs. (%)	103 (33.99)	187 (35.48)	0.72

TABLE 2. Clinical Characteristics of Groups Stratified by Presence of Chronic Stressors

Parameter	Presence of chronic stressors			Absence of chronic stressors		
	Group 1 (n = 103)	Group 2 (n = 187)	p	Group 1 (n = 200)	Group 2 (n = 340)	p
Men/women, abs. (%)	58/45 (56.31/43.69)	105/82 (56.15/43.85)	0.99	143/57 (71.50/28.50)	217/123 (63.82/36.18)	0.08
BMI, kg/m^2	31.74 ± 5.59	31.46 ± 4.98	0.98	29.99 ± 4.36	30.79 ± 5.11	0.65
Age, years	60.11 ± 7.42	59.07 ± 8.64	0.18	59.30 ± 8.01	58.74 ± 7.68	0.38
Systolic BP, mmHg	193.05 ± 22.27	177.06 ± 13.31	0.001	189.54 ± 19.13	175.43 ± 13.56	0.001
Diastolic BP, mmHg	111.26 ± 17.44	104.15 ± 9.65	0.001	107.13 ± 15.67	103.64 ± 10.02	0.002
TCH, mM	6.63 ± 1.42	6.28 ± 1.88	0.71	5.74 ± 1.33	5.69 ± 1.49	0.65
HDL CH, mM	1.98 ± 0.86	1.88 ± 0.56	0.74	1.77 ± 0.67	1.67 ± 0.61	0.86
LDL CH, mM	4.69 ± 1.63	4.28 ± 0.97	0.53	3.77 ± 1.01	3.82 ± 0.74	0.77
TG, mM	2.85 ± 1.14	2.65 ± 0.83	0.66	3.31 ± 1.00	2.29 ± 0.96	0.97

ic in 72 patients (23.76%), the hemodynamic in 78 patients (25.74%), and the lacunar in 29 patients (9.57%). There were no patients with arterioarterial emboli or strokes of the hemorheological microocclusion type. The study cohort consisted of unrelated people of Russian nationality, natives of the Russian Central Chernozem [8]. *Inclusion criteria* for the groups analyzed were a systolic arterial blood pressure (BP) ≥ 140 mmHg and/or diastolic BP ≥ 90 mmHg, and use by patients of antihypertensive drugs; *exclusion criteria* were the presence of symptomatic and secondary hypertension, kidney or liver failure, and refusal to take part in the study. The diagnosis "ischemic stroke" was made on the basis of data on neurological status, the patient's complaints, and brain CT/MRI scans. HTD was diagnosed in accordance with the diagnostic criteria of the All-Russian Scientific Cardiology Society [9]. Body mass index (BMI, kg/m^2) was determined for all subjects taking part in the study, along with total cholesterol (TCH), high-density lipoprotein cholesterol (HDL CH), low-density lipoprotein cholesterol (LDL CH), and triglycerides (TG). Blood samples for biochemical analysis were collected after 8-h starvation; analyses were run at the St. Joseph BDCH. All respondents assessed the presence of chronic stressors which, according to [10], included the following psychosocial factors: frequent stressful situations at home and/or at work, the absence of social support, family situation (single, separated, widowed), unsatisfactory social-economic status.

The characteristics of the study groups are given in Tables 1 and 2. There were no differences in age and sex composition, BMI, levels of TCH and LDL CH between patients of groups 1 and 2, or levels of stress experienced, though there were significant differences in BP, TG, and HDL CH ($p < 0.05$).

TABLE 3. Distribution of Polymorphic *MMP* Genotype Markers in the Study Groups

Locus	Genotype	Group 1 (n = 303)	Group 2 (n = 527)	OR (95% CI)	p
		abs. (%)			
rs11568818 MMP7	AA (ref)	113 (38.17)	179 (34.16)	1.00 (ref)	–
	AG	143 (48.31)	258 (49.24)	0.88 (0.63–1.21)	0.46
	GG	40 (13.52)	87 (16.60)	0.73 (0.46–1.16)	0.19
rs1320632 MMP8	AA (ref)	245 (82.49)	441 (84.64)	1.00 (ref)	–
	AG	48 (16.16)	76 (14.59)	1.14 (0.75–1.72)	0.59
	GG	4 (1.35)	4 (0.77)	1.80 (0.38–3.62)	0.64
rs11225395 MMP8	CC (ref)	97 (32.12)	163 (30.99)	1.00 (ref)	–
	CT	146 (48.34)	258 (49.05)	0.95 (0.68–1.33)	0.82
	TT	59 (19.54)	105 (19.96)	0.94 (0.62–1.44)	0.86
rs1799750 MMP1	1G/1G (ref)	85 (28.06)	153 (29.03)	1.00 (ref)	–
	1G/2G	146 (48.18)	258 (48.96)	1.02 (0.72–1.44)	0.98
	2G/2G	72 (23.76)	116 (22.01)	1.12 (0.74–1.69)	0.65
rs3025058 MMP3	6A/6A (ref)	101 (33.33)	149 (28.38)	1.00 (ref)	–
	5A/6A	148 (48.85)	263 (50.10)	0.83 (0.59–1.16)	0.30
	5A/5A	54 (17.82)	113 (21.52)	0.71 (0.46–1.08)	0.12
rs652438 MMP12	AA (ref)	259 (85.76)	460 (87.45)	1.00 (ref)	–
	AG	41 (13.58)	63 (11.98)	1.16 (0.74–1.80)	0.57
	GG	2 (0.66)	3 (0.57)	1.18 (0.14–1.73)	0.99
rs243865 MMP2	CC (ref)	170 (56.11)	311 (59.01)	1.00 (ref)	–
	CT	109 (35.97)	184 (34.92)	1.08 (0.79–1.48)	0.65
	TT	24 (7.92)	32 (6.07)	1.37 (0.75–2.49)	0.33
rs17577 MMP9	GG (ref)	200 (67.12)	352 (67.30)	1.00 (ref)	–
	AG	90 (30.20)	155 (29.64)	1.02 (0.73–1.41)	0.95
	AA	8 (2.68)	16 (3.06)	0.88 (0.34–2.22)	0.94

In both groups of patients – those subjected to stress and those not experiencing chronic stress – patients with stroke on the background of HTD and with HTD but not stroke were comparable in terms of sex, age, BMI, and lipid profile ($p > 0.05$). Study patients in both groups were significantly different in terms of BP ($p < 0.05$).

Genetic analysis. All patients were genotyped at eight polymorphic loci: rs1799750 of MMP1, rs243865 of MMP2, rs3025058 of MMP3, rs11568818 of MMP7, rs1320632 of MMP8, rs11225395 of MMP8, rs17577 of MMP9, and rs652438 of MMP12. Study polymorphisms were selected on the basis of previously described criteria [11]. All polymorphic markers selected for study were characterized by significant regulatory potential and influences on gene expression (as per the HaploReg (v. 4.1) database at http://archive.broadinstitute.org).

Venous blood (5-ml samples) was collected from the median cubital vein into plastic Vacutainer tubes with EDTA. Genomic DNA was extracted from peripheral blood leukocytes using a standard phenol-chloroform procedure. Polymorphisms were analyzed using the polymerase chain reaction (real-time PCR) on a CFX-96 real-Time System thermal cycler (Bio-Rad, USA) using oligonucleotide primers and probes synthesized at the Sintol Company. The nucleotide sequences of primers and probes are given by Lievre et al. and Pradhan-Palikhe et al. [12, 13]. Repeat genotyping of 5% of samples selected at random from patients of the study group demonstrated 100% reproducibility.

Assessment of the functional significance of polymorphisms. The regulatory potential of SNP was determined using the online HaploReg (v4.1) (https://pubs.broadinstitute.org) service. Associations of polymorphic alleles with the affinity of the DNA motif for transcription factors were assessed in terms of differences between LOD scores for the alternative (alt) and reference (ref) alleles [14]. The effects of SNP on the expression of genes (*cis*-eQTL) were analyzed using data from the Genotype-Tissue Expression project (GTEx) (http://www.gtexportal.org). Associations between alleles and levels of gene transcription were determined using the linear regression β coefficient, which characterizes changes in the normalized measures of gene expression on a single alt allele. Results with $p < 8 \cdot 10^{-5}$, $p_{FDR} \leq 0.05$ were used [15].

The study was approved by the Ethics Committee of the medical institute of Belgorod National State Research University; informed consent was obtained from all subjects.

Statistical analysis. Correspondence of genotype frequencies with the Hardy–Weinberg equilibrium was assessed using the χ^2 test. Allele and genotype frequencies in groups of patients with stroke and without stroke were analyzed using 2 × 2 linkage tables and the χ^2 test with the Yates correction for continuity. Calculations were run in Statistica for Windows 10.0. The nature of associations between SNP and stress with formation of stroke on the background of HTD was assessed using the odds ratio (OR) and its 95% confidence interval (95% CI), and differences were regarded as significant at $p < 0.05$.

Results and discussion. Distributions of allele and genotype frequencies in groups 1 and 2 for all the polymorphisms studied corresponded to the Hardy–Weinberg equilibrium ($p > 0.05$). The analysis results are presented in Table 3; there were no significant differences between study groups ($p > 0.05$).

Results from analysis of the interaction between *MMP* and chronic stresses on formation of ischemic stroke on the background of GP are shown in Table 4.

It is of note that formation of stroke on the background of HTD in people subjected to stress and patients not exposed to chronic stresses involved several polymorphic *MMP* loci. Among patients with ischemic stroke and subjected to regular stress, the frequency of GG homozygotes at the *MMP7* rs11568818 locus was significantly greater ($\chi^2 = 3.89$, $p = 0.04$) than in the group of patients with HTD without stroke. Thus, the G allele of rs11568818 increased the risk of developing stroke in patients with HTD and exposed to stress (OR 1.71, 95% CI 1.00–2.92). In the group of patients with HTD not exposed to chronic stress, the frequencies of the 5A allele ($\chi^2 = 5.43$; $p = 0.02$) and the 5A/5A genotype ($\chi^2 = 4.34$; $p = 0.04$) at the *MMP3* rs3025058 locus were significantly higher than among patients with stroke. These genetic variants had protective influences on formation of ischemic stroke on the background of HTD (OR 0.73 and OR 0.60, respectively). The gene-environment interactions seen here for polymorphic *MMP* loci and chronic stressors on formation of stoke may be due to the ability of matrix metalloproteinases to carry out the proteolytic cleavage of cell adhesion molecules, which modulate neuron plasticity and play a key role in stress-induced adaptations [16]. The important modifying influence of the stress factor on development of ischemic stroke has also been demonstrated in other studies. The results of six cohort studies (total 138,782 participants) were used to analyze a group of scientists in the People's Republic of China; this provided evidence that regular stress at work is associated with a high risk of developing ischemic stroke (OR 1.58, 95% CI 1.12–2.23) [5]. Published data indicate that chronic stress induces impairments to the operation of the hypothalamo-hypophyseal-adrenal axis and imbalance in sympathetic and parasympathetic regulation, which leads to the development of cerebrovascular pathology [7].

Analysis of the functional significance of polymorphic loci associated with stroke showed that the rs11568818 SNP of the *MMP7* gene is located in an area hypersensitive to DNase 1 in endotheliocytes, neuroglia, hematopoietic stem cells, fibroblasts, and normal and pathologically altered epithelial cells. This locus relates to the binding site for modified histone proteins (H3K4me1 and H3K4me3), which mark promoters in four tissues (including hematopoietic and mesenchymal stem cells, astrocytes, and neuron precursors) and enhancers in seven tissues (including the substantia nigra, caudate nucleus, and hippocampus). The rs11568818 SNP is located in the region attaching regulatory proteins with c-Fos, c-Jun, and TBP, and is also present in regulatory DNA motifs binding four transcription factors. The G gene variants at this locus, which is associated with a high risk of developing stroke, increases affinity for the transcription factors Foxa known-1 (ΔLOD score –3.1), PLZF (ΔLOD score –1.5), and Pou5fl known-2 (ΔLOD score –3.2) and decreases affinity for GR known4 (ΔLOD score 0.8) (https://pubs.broadinstitute.org). In silico analysis established that the "risky" G allele of the *MMP7* rs11568818 locus is associated with a decrease in the level of expression of the corresponding gene in the pancreas ($\beta = -0.31$, $p = 5.1 \cdot 10^{-11}$, FDR ≤ 0.05), lungs ($\beta = -0.35$, $p = 8.1 \cdot 10^{-14}$, FDR ≤ 0.05), skin ($\beta = -0.23$, $p = 3.9 \cdot 10^{-8}$, FDR ≤ 0.05), stomach ($\beta = -0.32$, $p = 1.9 \cdot 10^{-6}$, FDR ≤ 0.05), and liver ($\beta = -0.46$, $p = 1.2 \cdot 10^{-5}$, FDR ≤ 0.05). According to the GeneCards database, the *MMP7* gene is located on chromosome 11 and encodes a proteolytic enzyme cleaving elastin, types I, III, IV, and V gelatin, and fibronectin. *MMP7* proteinase is involved in remodeling the extracellular matrix, cell proliferation, and regeneration of damaged tissues, and supports cell migration and apoptosis (http://www.genecards.org/). The epigenetic effects of the polymorphic locus of the *MMP7* gene and the function of the corresponding peptidase may underlie the known association with formation of ischemic stroke. The literature available to us contained of association studies addressing

TABLE 4. Comparative Analysis of *MMP* Polymorphism Allele and Genotype Frequencies Stratified for the Presence of Stress

Locus	Allele, genotype	Presence of chronic stressors				Absence of chronic stressors			
		Group 1, abs. (%) (n = 103)	Group 2, abs. (%) (n = 187)	OR (95% CI)	p	Group 1, abs. (%) (n = 200)	Group 2, abs. (%) (n = 340)	OR (95% CI)	p
MMP1 rs1799750	1G	110 (53.40)	197 (52.67)	1.03 (0.72–1.47)	0.93	206 (51.50)	367 (53.97)	0.91 (0.70–1.16)	0.47
	2G	96 (46.60)	177 (47.33)	0.97 (0.68–1.39)		194 (48.50)	313 (46.03)	1.10 (0.86–1.43)	
	1G/1G	32 (31.07)	58 (31.02)	1.01 (0.57–1.74)	0.99	53 (26.50)	95 (27.94)	0.93 (0.61–1.40)	0.79
	1G/2G	46 (44.66)	81 (43.31)	1.05 (0.63–1.76)	0.92	100 (50.00)	177 (52.06)	0.92 (0.64–1.33)	0.71
	2G/2G	25 (24.27)	48 (25.67)	0.93 (0.51–1.68)	0.90	47 (23.50)	68 (20.00)	1.23 (0.79–1.91)	0.39
MMP2 rs243865	C	156 (75.73)	281 (75.13)	1.03 (0.68–1.56)	0.95	293 (73.25)	525 (77.21)	0.80 (0.60–1.09)	0.16
	T	50 (24.27)	93 (24.87)	0.97 (0.64–1.46)		107 (26.75)	155 (22.79)	1.24 (0.92–1.66)	
	CC	61 (59.22)	106 (56.68)	1.11 (0.66–1.86)	0.77	109 (54.50)	205 (60.29)	0.79 (0.55–1.14)	0.22
	CT	34 (33.01)	69 (36.90)	0.84 (0.49–1.44)	0.59	75 (37.50)	115 (33.83)	1.17 (0.80–1.72)	0.44
	TT	8 (7.77)	12 (6.42)	1.22 (0.44–3.37)	0.85	16 (8.00)	20 (5.88)	1.39 (0.67–2.88)	0.44
MMP3 rs3025058	5A	92 (44.66)	161 (43.04)	1.06 (0.74–1.52)	0.77	164 (41.00)	328 (48.52)	0.73 (0.57–0.95)	**0.02**
	6A	114 (55.34)	213 (56.95)	0.94 (0.65–1.34)		236 (59.00)	348 (51.48)	1.36 (1.05–1.75)	
	5A/5A	22 (21.36)	32 (17.11)	1.31 (0.69–2.51)	0.46	32 (16.00)	81 (23.97)	0.60 (0.37–0.97)	**0.04**
	5A/6A	48 (46.60)	97 (51.87)	0.81 (0.48–1.34)	0.46	100 (50.00)	166 (49.11)	1.04 (0.72–1.49)	0.91
	6A/6A	33 (32.04)	58 (31.02)	1.05 (0.60–1.82)	0.96	68 (34.00)	91 (26.92)	1.39 (0.94–2.08)	0.10
MMP7 rs11568818	A	64 (32.65)	152 (40.86)	0.70 (0.48–1.02)	0.07	145 (36.62)	282 (41.71)	0.81 (0.62–1.05)	0.11
	G	132 (67.35)	220 (59.14)	1.42 (0.97–2.08)		251 (63.38)	394 (58.29)	1.24 (0.95–1.61)	
	AA	10 (10.20)	26 (13.98)	0.70 (0.29–1.60)	0.47	23 (11.62)	61 (18.05)	0.60 (0.34–1.03)	0.06
	AG	44 (44.90)	100 (53.76)	0.70 (0.42–1.18)	0.19	99 (50.00)	160 (47.34)	1.11 (0.77–1.60)	0.61
	GG	44 (44.90)	60 (32.26)	1.71 (1.00–2.92)	**0.04**	76 (38.38)	117 (34.61)	1.17 (0.80–1.72)	0.43
MMP8 rs1320632	A	178 (89.00)	342 (92.43)	0.66 (0.35–1.24)	0.22	360 (90.91)	616 (91.67)	0.91 (0.57–1.44)	0.75
	G	22 (11.00)	28 (7.57)	1.51 (0.80–2.81)		36 (9.09)	56 (8.33)	1.10 (0.69–1.74)	
	AA	80 (80)	159 (85.94)	0.65 (0.32–1.30)	0.26	165 (83.33)	282 (83.93)	0.96 (0.58–1.58)	0.95
	AG	18 (18)	24 (12.98)	1.47 (0.72–3.01)	0.33	30 (15.15)	52 (15.48)	0.97 (0.58–1.63)	0.99
	GG	2 (2)	2 (1.08)	1.87 (0.18–3.90)	0.92	3 (1.52)	2 (0.59)	2.57 (0.34–2.09)	0.55
MMP8 rs11225395	C	113 (54.85)	205 (54.81)	1.00 (0.70–1.43)	0.99	227 (57.04)	379 (55.90)	1.05 (0.81–1.35)	0.76
	T	93 (45.15)	169 (45.19)	0.99 (0.69–1.42)		171 (42.96)	299 (44.10)	0.95 (0.74–1.24)	
	CC	27 (26.22)	59 (31.55)	0.77 (0.43–1.36)	0.41	70 (35.17)	104 (30.68)	1.23 (0.83–1.81)	0.32
	CT	59 (57.28)	87 (46.52)	1.54 (0.92–2.58)	0.10	87 (43.72)	171 (50.44)	0.76 (0.53–1.10)	0.15
	TT	17 (16.50)	41 (21.93)	0.70 (0.35–1.37)	0.34	42 (21.11)	64 (18.88)	1.15 (0.73–1.82)	0.61
MMP9 rs17577	G	164 (82.83)	311 (84.51)	0.88 (0.54–1.44)	0.16	326 (81.91)	548 (80.83)	1.07 (0.77–1.50)	0.72
	A	34 (17.17)	57 (15.49)	1.13 (0.69–1.84)		72 (18.09)	130 (19.17)	0.93 (0.67–1.30)	
	GG	66 (66.67)	133 (72.28)	0.76 (0.44–1.35)	0.39	134 (67.34)	219 (64.60)	1.13 (0.77–1.66)	0.58
	GA	32 (32.32)	45 (24.46)	1.47 (0.83–2.62)	0.20	58 (29.14)	110 (32.45)	0.86 (0.57–1.28)	0.48
	AA	1 (1.01)	6 (3.26)	0.30 (0.01–2.58)	0.45	7 (3.52)	10 (2.95)	1.20 (0.40–3.48)	0.91
MMP12 rs652438	A	189 (91.75)	353 (94.39)	0.66 (0.32–1.35)	0.29	370 (92.50)	630 (92.65)	0.98 (0.60–1.61)	0.99
	G	17 (8.25)	21 (5.61)	1.51 (0.74–3.07)		30 (7.50)	50 (7.35)	1.02 (0.62–1.67)	
	AA	87 (84.47)	167 (89.31)	0.65 (0.30–1.40)	0.31	172 (86.00)	293 (86.18)	0.98 (0.58–1.68)	0.99
	AG	15 (14.56)	19 (10.16)	1.51 (0.69–3.29)	0.36	26 (13.00)	44 (12.94)	1.01 (0.58–1.74)	0.99
	GG	1 (0.97)	1 (0.53)	1.82 (0.05–7.04)	0.99	2 (1.00)	3 (0.88)	1.13 (0.13–8.40)	0.99

Significant differences are shown in bold.

analysis of the involvement of the rs11568818 SNP and the development of ischemic stroke, though there are reports of associations between this locus and cardiovascular pathology in people living in Sweden [17].

The rs3025058 single-nucleotide polymorphism of the *MMP3* gene is indicated by HaploReg (ver4.1) to be located in the region of histone H3K4me1, which encodes enhancers in mesenchymal cells and epitheliocytes. This locus is located in an area hypersensitive to DNase 1 in the thymus and in the area of regulatory DNA motifs binding two transcription factors. The 6A allele has been found to decrease affinity for transcription factors Ciz (ΔLOD score 1.3) and Gfi-1b (ΔLOD score 1.7). According to the GeneCards database, he *MMP3* gene encodes enzyme MMP3, which is able to hydrolyze fibronectin, laminin, proteoglycans, collagens, and types I–V gelatin (http://www.genecards.org/). The level of *MMP3* expression is regulated by cytokines and growth factors and is increased in atherogenesis, oncogenesis, and post-injury tissue regeneration. The results obtained here are consistent with data from other association studies: Sherva et al. [18] found that Americans with the 5A/5A genotype at the rs3025058 locus of the *MMP3* gene have a significantly lower risk of developing ischemic stroke than carriers of the 5A/6A and 6A/6A genotypes (OR 0.51, 95% CI 0.31–0.85, $p = 0.01$).

Thus, the results of the studies reported here provide evidence for the significant role of the interaction of polymorphic MMP loci with stress in the formation of ischemic stroke on the background of HTD. Risk factors for disease development in patients subjected to chronic stressors are the rs11568818 GG genotype of the *MMP7* gene (OR 1.71). The 5A allele has protective value in relation to the development of stroke in people not subjected to chronic stressors (OR 0.73), as does the 5A/5A genotype (OR 0.60) of the rs3025058 polymorphism of the *MMP3* gene. These polymorphic loci are characterized by significant regulatory potential (they are located in the area of histones marking promoters and enhancers in cell cultures, neuron precursors, mesenchymal cells, etc., and the area containing regulatory DNA motifs and binding sites for transcription factors), while the rs11568818 SNP is linked with the level of expression of the *MMP7* gene in the liver, lungs, pancreas, and other organs.

The authors have no conflicts of interests.

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